

- c. 'Analyte' is the analyte or an analyte-related reactant, and subsequently
- ii. determining a detectable signal from Reactant\* in the complex (sample value), and
- iii. obtaining the amount of analyte in the sample by comparing the sample value with one or more calibrator values, each of which corresponds to a standard amount of analyte,

wherein A) before determination of the calibrator value, either (i) calibrator or (ii) a binder for the calibrator has been bound to a matrix, and when a binder for the calibrator has been bound to the matrix, calibrator is added or calibrator predeposited in the matrix is released at the determination of calibrator value, and wherein the matrix is insoluble in the liquid medium in which binding of Reactant\* to the calibrator occurs, B) the calibrator and the analyte are capable of biospecifically binding to Reactant\* by equivalent binding sites, and C) one or more calibrator zones CZ comprising calibrator or binder for the calibrator are located in a single process flow stream with Reactant I in a detection zone (DZ).

11. (Third Amendment) The method according to claim 1, wherein along a single matrix is the flow matrix, and wherein along a single process flow stream, there are

- a. one or more calibrator zones (CZ), each of which exhibits a matrix calibrator or a matrix calibrator binder,
- b. one or more detection zones (DZ), none of which coincides with any calibrator zone, and in which a Capturer is firmly anchored and is either Reactant I or a biospecific affinity reactant, which directly or indirectly binds Reactant I biospecifically,
- c. an application zone for Reactant\*,  $A_R \cdot Z$ , which is located upstream of said CZ and DZ and to which Reactant\* is optionally predeposited, and
- d. an application zone for sample ( $A_S Z$ ) which is located

- i. upstream of or coinciding with a detection zone,
- ii. downstream or upstream of or coinciding with  $A_R \cdot Z$  ( $A_S Z / A_R \cdot Z$ ), or
- iii. upstream of, downstream of or coinciding with a calibrator zone,

wherein Reactant\* is added to  $A_R \cdot Z$  if Reactant\* is not predeposited, or buffer is added to  $A_R \cdot Z$  if Reactant\* is predeposited, and sample is added to  $A_S Z$ , optionally premixed with Reactant\* if  $A_S Z$  and  $A_R \cdot Z$  coincide, such that analyte and Reactant\* reach DZ at the same time, or such that analyte reaches DZ before Reactant\*.

15. (Third Amendment) The method according to claim 11, wherein

- a.  $A_S Z$  is (i) common to  $A_R \cdot Z$ , forming a common zone ( $= A_S Z / A_R \cdot Z$ ) or (ii) is located upstream of  $A_R \cdot Z$ , and
- b. for alternative (i) sample is premixed with Reactant\* before it is added to the common zone  $A_S Z / A_R \cdot Z$ , or sample is added to the common zone  $A_S Z / A_R \cdot Z$  containing predeposited Reactant\*, or for alternative (ii), sample is added to  $A_S Z$ , which is located upstream of  $A_R \cdot Z$  which in turn comprises predeposited Reactant\*.

23. (Twice Amended) The device according to claim 20, wherein the process flow comprises a detection zone (DZ) which is located downstream of  $A_R \cdot Z$  and comprises a firmly anchored Capturer via which Reactant\* can bind to DZ, and a zone of application of sample ( $A_S Z$ ) which is located upstream of or coincides with said DZ.